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Escherichia coli Survival in, and Release from, White-Tailed Deer Feces

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White-tailed deer are an important reservoir for pathogens that can contribute a large portion of microbial pollution in fragmented agricultural and forest landscapes. The scarcity of experimental data on survival of microorganisms in and release from deer feces makes prediction of their fate and transport less reliable and development of efficient strategies for environment protection more difficult. The goal of this study was to estimate parameters for modeling *Escherichia coli* survival in and release from deer (*Odocoileus virginianus*) feces. Our objectives were as follows: (i) to measure survival of *E. coli* in deer pellets at different temperatures, (ii) to measure kinetics of *E. coli* release from deer pellets at different rainfall intensities, and (iii) to estimate parameters of models describing survival and release of microorganisms from deer feces. Laboratory experiments were conducted to study *E. coli* survival in deer pellets at three temperatures and to estimate parameters of Chick's exponential model with temperature correction based on the Arrhenius equation. Kinetics of *E. coli* release from deer pellets were measured at two rainfall intensities and used to derive the parameters of Bradford-Schijven model of bacterial release. The results showed that parameters of the survival and release models obtained for *E. coli* in this study substantially differed from those obtained by using other source materials, e.g., feces of domestic animals and manures. This emphasizes the necessity of comprehensive studies of survival of naturally occurring populations of microorganisms in and release from wildlife animal feces in order to achieve better predictions of microbial fate and transport in fragmented agricultural and forest landscapes.

Fecal bacteria are a leading cause of water body impairment in the United States (1), and *Escherichia coli* is currently used by the Environmental Protection Agency (EPA) as an indicator organism for fecal contamination and bacterial impairment for watersheds. Currently, there are 3,451 impaired water bodies in the United States, based on *E. coli* monitoring (1). It has been recognized that livestock and wastewater are major sources of fecal contamination in watersheds (2–4), while cattle are commonly considered a principal reservoir of *E. coli* O157:H7 (5–7). However, in fragmented agricultural and forest landscapes, wildlife can contribute a large portion of the fecal pollution (8–11), also serving as a reservoir for pathogens. *E. coli* O157:H7 was found in feral swine in Sweden (12) and in California, USA (13–15); *E. coli* O157:H7 were also isolated from feces of white-tailed deer cograzing with cattle (16–18). Black-tailed deer were identified as a source of *E. coli* O157:H7 strawberry-transmitted infection in Oregon, which caused 15 illness cases, including two deaths (19). Seventy people were reported to be infected by *E. coli* O157:H7 after consumption of unpasteurized apple juice in the western United States and British Columbia, Canada, in October 1996. This outbreak of *E. coli* O157:H7 infection was suspected to be associated with apples coming from orchards frequented by deer (20). Since *E. coli* O157:H7 is spread via a fecal-oral route and deer may harbor this pathogen, there is a possibility for other ruminants to become infected through exposure to contaminated feces (21).

Increasing microbial pollution associated with livestock operations and wildlife stimulated research of microbial survival in fecal materials, manure-amended soils, bottom sediment, and open bodies of water that has been summarized in several comprehensive reviews (22–25). Temperature, water content, and pH were suggested to be the most important factors that control bacterial survival. Reddy et al. (26) and Mubiru et al. (27) demonstrated that increases in water content caused a decrease in microbial die-off rates in soil. Shorter survival of enteric bacteria was reported in acid soils (28, 29). An increase in temperature was shown to increase growth rates of *E. coli* in river water (30) but reduce survival duration (31, 32).

The majority of previous work focused on *E. coli* from poultry and dairy sources, including manure slurries from dairy facilities and fresh cattle deposits. Less attention was devoted to survival of *E. coli* originating from wildlife. Specifically, Gallagher et al. (4) studied survival of *E. coli* originating from feral hogs and deer and isolated from soil and creek water, and survival of *E. coli* introduced into streambed sediments from goose and deer feces was examined by Kiefer et al. (33). Surprisingly, despite recognition that wildlife can potentially contribute *E. coli* contamination in the environment, to the best of our knowledge there are no published reports of *E. coli* survival in wildlife fecal material.

The survival of *E. coli* in feces is only one side of the coin; the other side is *E. coli* release from fecal material. Indeed, unless the bacteria are directly deposited into water bodies, only a fraction of surviving *E. coli* that is released from fecal deposits with rainfall water can be transported with runoff and contaminate surface water. Several works dealt with microbial release from manure or fecal deposits. Springer et al. (34) were probably the first to sys-

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tematically address the release of fecal coliforms from artificial cowpats using a rain simulator and showed the effect of manure age and rainfall intensity on fecal coliform release. The effect of manure age in artificial cowpats was further studied by Thelin and Gifford (35), Kress and Gifford (36), and Muirhead et al. (37), whose results indicated that numbers of released bacterial cells correlated with declining bacterial concentrations in cowpats due to die-off. Additional experiments on kinetics of bacterial release from cattle manure reported differences in release kinetics for different manure components (38–40) and revealed the effects of manure type, temperature, and solution salinity on the release kinetics (41, 42). These experiments also provided parameters for the event-based model KINEROS2/STWIR (43–45), which predicts overland transport of bacteria released from fecal deposits or surface-applied manure. All listed studies used bovine manure or fecal deposits, and to the best of our knowledge, no attempts were undertaken to quantify release of *E. coli* from fecal material produced by wildlife.

This study aimed to fill a gap in this knowledge and provide parameters for modeling *E. coli* survival and release from wildlife fecal material. We chose white-tailed deer (*Odocoileus virginianus*) pellets as a source of *E. coli*. The objectives of this study were as follows: (i) to measure survival of *E. coli* in deer pellets at different temperatures, (ii) to measure kinetics of *E. coli* release from deer pellets at different rainfall intensity, and (iii) to estimate parameters of models describing survival and release of microorganisms from fecal material.

MATERIALS AND METHODS

Survival experiment. Freshly deposited fecal pellets were collected from three female white-tailed deer (*Odocoileus virginianus*) maintained at the Michigan State University Veterinary Research Farm. Samples were transported to the laboratory, and individual pellets were placed in sterile specimen containers. The specimen containers were divided into three lots. One lot each was stored at 4°C, 20°C, and 35°C. Two pellets were removed from each storage condition and assayed for *E. coli* on days 1, 2, 4, 8, 13, 16, 19, and 31 after collection. A total of 56 pellets were used in *E. coli* assays. On each day, only two pellet replications were used at each temperature. The reason is that only fresh excreted material could be used for assays in order to avoid an influence of the differences in pellet compositions, pellet properties, and times between deposition and the start of incubation experiments on bacterial survival. Between measurements, all pellets were stored in closed sterile specimen containers to prevent water loss via evaporation. Fecal pellets were individually weighed to determine wet weight in sterile Whirl-Pak (Nasco, WI) bags. Sterile phosphate-buffered water (46) (100 ml) was added to each bag, and the pellets were homogenized for 5 min in a BagMixer 400P stomacher (Interscience, France) at 8 strokes per second. Forty milliliters of the homogenized pellet suspension was centrifuged at 4°C in a sterile centrifuge tube at 1,000 × *g* for 15 min to separate large debris. The supernatant was transferred into a sterile tube and serially diluted with sterile phosphate-buffered water. Dilutions were processed by membrane filtration, and membranes were transferred to mTEC (Becton, Dickinson and Company, Sparks, MD) agar plates. Two plates for each pellet were placed in waterproof bags and incubated at 35 ± 0.5°C for 1 h and then submerged in a water bath at 44.5 ± 0.2°C for 22 to 24 h. After incubation, magenta colonies were counted and adjusted by the processed volume to determine *E. coli* concentration per unit of pellet wet weight. In addition to *E. coli* content measurements, dry mass and water content were measured in pellets from each group. These measurements were used to calculate *E. coli* content per unit of dry mass of the pellet.

The inactivation of *E. coli* in the deer pellet was described using Chick's (47) exponential model with temperature correction based on the Arrhenius equation:

$$-\frac{dC}{dt} = \mu C \qquad \mu(T) = \mu_r \lambda^{(T-T_r)} \tag{1}$$

where C is the bacterial content in the pellet (CFU g of dry pellet^{-1}), t is time (day), T is the temperature (°C), μ is the first-order inactivation rate constant (day^{-1}), λ is the temperature correction coefficient (dimensionless), and T_r and μ_r are the reference temperature and the bacterial inactivation rate at T_r.

The inactivation parameters λ and μ_r for *E. coli* were obtained by fitting equation 1 to the experimental data using a regression wizard of the SigmaPlot software (Systat Software, San Jose, CA, USA). Equation 1 was applied only to the declining limb of measured *E. coli* concentration dynamics in the pellet:

$$\ln C = \ln C_e - \mu(t - t_e) \tag{2}$$

where C_e is the *E. coli* concentration at the beginning of the exponential inactivation stage (CFU g of dry pellet^{-1}) and t_e is the time of the beginning of this stage (day).

Release experiment. Laboratory experiments were conducted to study the influence of rainfall and irrigation intensity on the release of *E. coli* from deer pellets. Fresh pellets from white-tailed deer (*Odocoileus virginianus*) were collected as described above and stored in a sterile screw-cap vials for 4 days at temperature of 4°C and 20°C to let *E. coli* approach the highest concentration in pellets. Then one pellet was placed on a metal screen mounted inside a 50-ml pipette and was subjected to irrigation. Deionized water was applied through a septum needle installed at a height of 56 cm above the pellet to mimic rain drops on the pellet surface. The needle was adjusted horizontally to drip on the center of the pellet. Six constant flow velocities ranging from 3.7 to 11.7 ml/h were maintained during the experiment by peristaltic pump (model RP-1; Dynamax; Rainin Instrument Co., Emeryville, CA). The effluent was collected from the pipette outlet and analyzed for *E. coli* content similarly to the survival experiment. The first portion of the effluent solution was taken 10 min after the peristaltic pump was turned on, and the other 11 samples were collected at 5-min intervals during the experiment. The experiment was conducted in a cold room at 4°C to minimize *E. coli* growth.

The measured release kinetics were used to compute the parameters of the Bradford-Schijven bacterial release model (41). The model was applied to the cumulative data of the released mass of bacteria to reduce model sensitivity to the oscillations in the concentration values:

$$M_t = \begin{cases} 0; & t \le t_i \\ M_0[1 - [1 + a\beta q(t - t_i)]^{-1/\beta}]; & t > t_i \end{cases} \qquad a = \alpha/q \tag{3}$$

where M_t is the cumulative number of bacterial CFU released into the aqueous phase within time t (CFU), M_0 is the initial number of bacterial CFU in the pellet (CFU), q is the water application rate (ml h^{-1}), t_i is the time when the release started (h), and α (h^{-1}) and β (dimensionless) are fitting parameters defining the shape of the release curve.

It has been shown (40) that model fitting to a single release curve does not provide reliable estimates of release parameters. Therefore, by following the approach suggested by Guber et al. (40), the Levenberg-Marquardt nonlinear least-squares algorithm was applied to the whole data set with the same values of the parameters (α and β) for all measured release curves but with M_0 values being individual for each curve. The initial M_0 values were estimated from *E. coli* measurements in the pellet taken from the same pellet group prior to irrigation and then corrected during model fitting to the experimental data. The goodness of fit for the model application was assessed by estimating the root mean square error (RMSE) between measured and simulated cumulative release curves.

RESULTS

Survival experiment. Two distinct stages in *E. coli* survival were observed in the survival time series. A relatively short bacterial

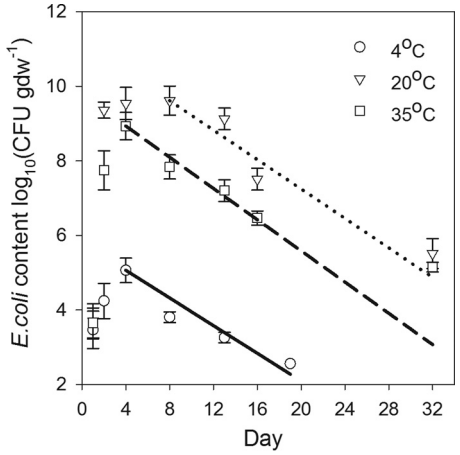

FIG 1 *E. coli* concentrations measured in deer pellets during the survival experiment at three studied temperatures. Lines represent results of fitting equation 2 to the bacterial die-off stage.

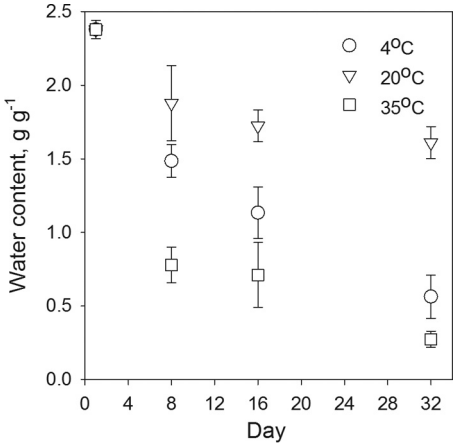

FIG 2 Changes in water content of deer pellets measured in the *E. coli* survival experiment at the three temperatures studied.

growth stage that lasted from 4 to 8 days was followed by a relatively long die-off stage. Detectable concentrations of *E. coli* were still present at the end of the experiment (Fig. 1). The *E. coli* content increased from 1.5 to 3 orders of magnitude during the first stage at three studied temperatures. Maximum *E. coli* growth was observed at temperature of 20°C, where the bacterial concentrations approached 9.62 $\log_{10}$(CFU g [dry weight]$^{-1}$) at the end of the growth stage. The least growth occurred at 4°C, which produced maximum *E. coli* concentration of 5.07 $\log_{10}$(CFU g [dry weight]$^{-1}$).

The duration of the first stage and the rates of *E. coli* growth were different for the three temperatures. The first stage lasted for 4 days at temperatures of 4°C and 35°C but was twice as long at 20°C. The bacterial growth was fastest between the first and second day of the experiment, with average daily rates of 0.776, 5.660, and 4.091 $\log_{10}$(CFU g [dry weight]$^{-1}$) for 4°C, 20°C, and 35°C, respectively. After growth peaked on day 4 at 4°C and 35°C, *E. coli* concentrations rapidly declined. However, at 20°C, *E. coli* concentrations remained close to the maximum value between day 2 and day 13 (Fig. 1) before entering the declining stage.

The second stage of the *E. coli* survival experiment was characterized by approximately the same die-off rates for the three temperature regimens, as indicated by the slope in the graphs of $\log_{10}$ *E. coli* concentration versus day in Fig. 1. This was consistent with the values of parameters obtained in the fitting of equation 2 to the declining limb of the survival time series. Specifically, the value of the temperature correction coefficient (λ) was close to unity (1.007), indicating a minor influence of temperature on *E. coli* die-off. The computed value of the bacterial inactivation rate (μ_r) was 0.175 day^{-1} at a reference temperature (T_r) of 20°C, while μ values were 0.158 and 0.194 day^{-1} for 4°C and 35°C, respectively. Values of R^2 (0.984) and RMSE of the *E. coli* concentrations {0.688 $\log_{10}$(CFU g [dry weight]$^{-1}$])} indicated acceptable accuracy of the fit of the model (equation 2) to the experimental data.

Water content measured in the deer pellets varied during the survival experiment. We observed a decrease in water content from initial values of 2.379 ± 0.062 g g^{-1} to the final values of 0.564 ± 0.147 g g^{-1}, 1.610 ± 0.109 g g^{-1}, and 0.274 ± 0.055 g g^{-1} during the 32 days of the survival experiment at 4°C, 20°C, and 35°C, respectively. The decrease was more pronounced during the first 8 days of the experiment (Fig. 2). Average daily water content losses during the first week of the experiment ranged from 0.072 to 0.229 g g^{-1} and were largest at 35°C and smallest at 20°C. Overall, pellet water content during the survival experiment decreased faster at 4°C than 20°C and 35°C.

In order to determine how *E. coli* survival in deer pellets differs from its survival in other environments, we compared the results of this research with multiple published studies of *E. coli* survival conducted at different temperatures and in different environments. The environments addressed in the reviewed published studies included bovine feces (48, 49), fresh dairy manure and slurry, old dairy slurry (50), sheep feces (51), sandy loam soil (52), loamy sand and sandy loam sediment (53), and stream water (54). Results of these studies were used to estimate the value of the first order inactivation rate constant at a reference temperature of 20°C (μ_{20}) and the value of the temperature correction coefficient (λ) in equation 1. The comparison of inactivation rates obtained for different environmental conditions showed that the μ_{20} value obtained in this study for deer pellets is the closest to that reported for bovine feces by Wang et al. (48) (Table 1). Among the exam-

TABLE 1 Parameters of *E. coli* inactivation model (equations 1 and 2) for different environments estimated from different data sources

Environment[a]	μ_{20} (day^{-1})	λ	R^2	Temperature range (°C)	Reference
Bovine feces*	0.168	1.045	0.942	5–37	48
Old dairy slurry*	0.124	1.078	0.998	4–37	50
Fresh dairy slurry*	0.081	1.083	0.984	4–37	50
Fresh dairy manure*	0.090	1.069	0.975	4–37	50
Sheep feces	0.242	1.095	0.792	5–18	51
Deer pellet	0.175	1.007	0.984	4–35	This study
Sandy loam soil	0.069	1.072	0.996	5–37	67
Loamy sand sediment	0.230	1.109	0.988	4–24	55
Sandy loam sediment	0.088	1.066	0.894	4–24	55
Stream water	0.859	1.108	0.976	5–25	54

[a] *, laboratory strains of *E. coli* O157:H7 were used. Naturally occurring *E. coli* was used in all other environments.

TABLE 2 Measured and computed variables and statistics of the *E. coli* release model (equation 3)

Irrigation rate (ml h^{-1})	Initial *E. coli* content in pellet: Per dry mass (10^8 CFU g [dry wt]$^{-1}$)[a]	Total cells (10^8 CFU)[a]	Fitted M_0 (10^8 CFU)	*E. coli* recovery (%)	RMSE (10^5 CFU)[b]
3.7	4.96, 8.06	1.00, 1.63	4.15	15.8	20.2
4.2	8.31, 13.9	1.13, 1.88	5.22	15.6	17.8
6.4	0.62, 1.24	0.12, 0.25	0.26	20.3	3.6
10.7	19.7, 23.2	4.18, 4.92	4.67	23.6	7.9
11.6	21.9, 25.9	5.65, 6.66	3.55	21.4	31.2
11.7	14.2, 17.5	3.35, 4.13	4.92	24.8	13.9

[a] Minimum and maximum values measured in replicates.
[b] Root mean square error of fitting equation 3 to the cumulative *E. coli* release curves.

ined studies, the smallest and largest μ_{20} values were obtained in sandy loam soil (67) and in stream water (54), respectively. Inactivation rates obtained for sediment by Garzio-Hadzick et al. (55) were higher or lower than the μ_{20} for deer pellets of our study depending on the sediment texture. We plotted *E. coli* die-off rates at different temperatures based on the data shown in Table 2 (Fig. 3). As can be seen from Fig. 3, the inactivation rate for deer pellets was nearly the same as that in stream water and considerably higher than that in the other environments at 4°C but was the lowest among all the environments at 35°C.

Release experiment. High variation of naturally occurring *E. coli* concentrations was observed in the collected deer pellets used for the release experiments. The initial concentrations spanned an order of magnitude of 1.6 (Table 2). High oscillations of *E. coli* concentrations were observed in the effluent during the whole experiment (Fig. 4). Overall, a gradual decrease in *E. coli* concentrations was observed at low irrigation rates (Fig. 4a), while an abrupt decrease occurred at irrigation rates of 11.6 and 11.7 ml h^{-1} after 40 min of irrigation (Fig. 4b). Visual inspection revealed holes in the pellet formed by droplets. *E. coli* concentrations in the effluent were the highest for the irrigation rate of 4.2 ml h^{-1} and the lowest for 6.4 ml h^{-1}.

Cumulative release curves had a smooth shape and a general increase in total numbers of released CFU with time was observed for all irrigation rates. More *E. coli* was released at high irrigation rates than at low rates (Fig. 5). The recovery of *E. coli* was also different at different irrigation rates: 15.8% to 20.3% of *E. coli* CFU were recovered from the pellets at low irrigation rates, while 21.4% to 24.8% were recovered at high rates (Table 2).

The Bradford-Schijven bacterial release model (equation 3) reproduced all experimental data reasonably well (Fig. 5). Root mean square errors ranged from 3.6×10^5 to 31.2×10^5 CFU; that is, they constituted less than 10% of the recovered culturable bacteria. Given the high variability of *E. coli* concentrations in replicated measurements, the model accuracy can be regarded as acceptable. Fitted values of the initial number of bacterial CFU in pellets (M_0) deviated slightly from measured values but also were within a reasonable range (Table 2). These deviations were rather random, since no correlation was found between the measured total CFU and the fitted values. The values of fitting parameters defining the shape of the release curves were as follows: $a = 0.0969$ ml^{-1} and $\beta = 1.924$ (Table 3).

DISCUSSION

Survival experiment. The two-stage kinetics of *E. coli* survival in deer pellets observed in this study was consistent with results published by other authors for fecal coliform growth and die-off in manure amended soil, sediment, and runoff water in early (35, 48–50, 53, 56–58) and recent studies (33, 55). Specifically for dairy manure, Himathongkham et al. (50) observed a slight increase in *E. coli* O157:H7 GFP population during the first 3 days followed by a 6-log decrease during subsequent 35 days of the experiment. Wang et al. (48) reported a 2-$\log_{10}$ increase of inoculated *E. coli* O157:H7 population in bovine feces after 2 days at 37°C. A 1.5- to 2.0-$\log_{10}$(CFU/g) increase in *E. coli* O157:H7 population was observed in dairy cattle feces during the first 3 days, followed by a 2- to 5-$\log_{10}$(CFU/g) decrease during the next 4 weeks of incubation (49). In our study, the first stage of *E. coli* increase lasted much longer (up to 8 days) and the increase was much more pronounced [up to 3 $\log_{10}$(CFU/g)] than in the published dairy studies. This implies that *E. coli* concentrations measured in fresh deer deposits cannot be used directly for informing total maximum

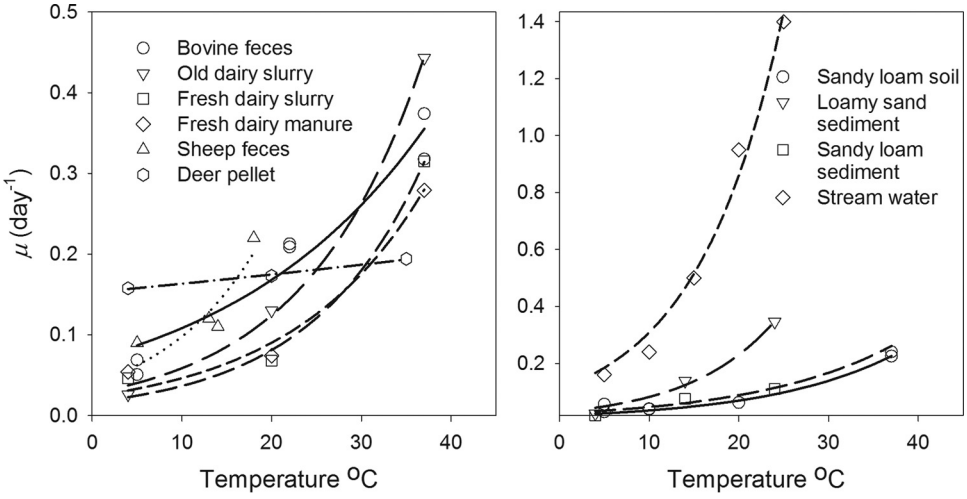

FIG 3 Relationships between *E. coli* die-off rates and temperature estimated for different environments based on parameters of *E. coli* inactivation model (equation 1 and equation 2) obtained from published studies listed in Table 2.

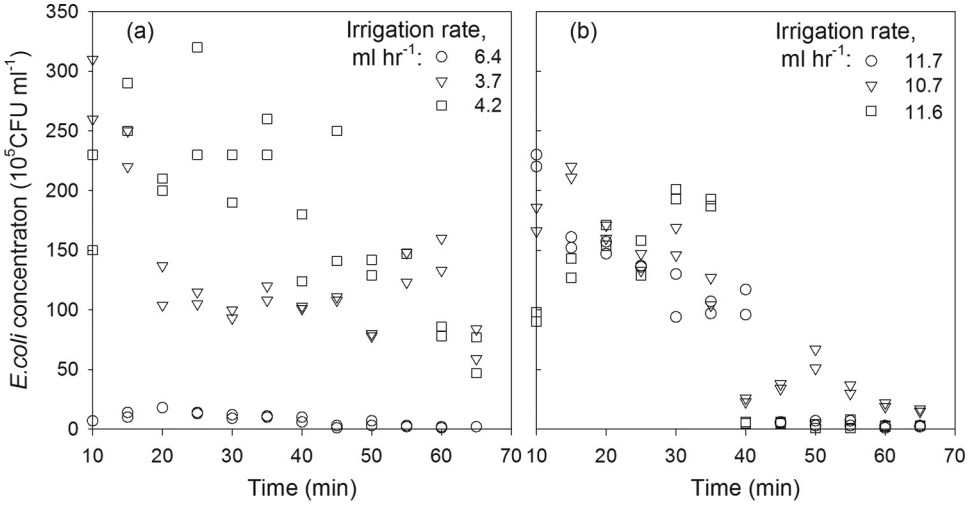

FIG 4 *E. coli* concentrations measured in the effluent during the release experiments with low (a) and high (b) irrigation rates.

daily load (TMDL) models, because this could lead to substantial underestimation of the *E. coli* inputs. Higher potential of *E. coli* growth in deer pellets should not be neglected during estimations of *E. coli* loads in watersheds.

The increase in *E. coli* concentration during the first stage of survival in the fecal depositions is often attributed to several factors. Among them are reduction of environmental stresses on the bacteria and lowered levels of toxic compounds in the growth medium due to dilution, increased nutrient supply, fewer antagonistic effects from other organisms, and changes in oxygen level in the new environment (23). In our study, the change in oxygen level was likely the primary factor among those listed above that affected the enhanced *E. coli* growth, since the pellets were not diluted and the nutrient supply for *E. coli* populations was unlimited.

Temperature is an important factor affecting bacterial growth (30, 59). Indeed, *E. coli* grew faster and maximum *E. coli* concentrations in deer pellets were higher at 20°C than at 4°C and 35°C. Minimum growth observed in our study at 4°C was consistent with results of earlier *E. coli* survival studies in different environments, i.e., dairy manure (50), cattle feces (49), and glucose minimal medium (60). The slow *E. coli* growth or absence of growth at low temperatures was commonly attributed to a limited ability of *E. coli* to synthesize protein (61–63). Contrary to some studies (e.g., reference 48), our results showed a 2-log increase in *E. coli* population at 4°C. The increase of *E. coli* growth in the deer pellet with temperature concurs with *E. coli* growth observed in cattle cowpats (64). Martinez et al. (64) found that the thermal day growth rate increased exponentially with temperature during the first week of bacterial sampling. However, in their study, temperatures below 15°C produced negative or zero *E. coli* growth, while in our study, the growth was positive. These differences in *E. coli* growth can be attributed partly to different *E. coli* strains and different growing conditions in the dairy cattle feces and cowpats used in previous studies compared to the white-tailed deer feces used in our study.

During the second survival stage, the highest *E. coli* concentrations in the pellets were observed at the highest water contents and

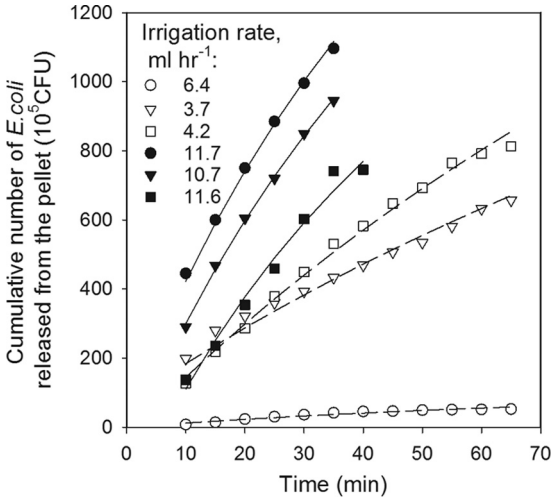

FIG 5 Measured (symbols) and simulated (lines) kinetics of *E. coli* release from deer pellets at low (open symbols) and high (closed symbols) irrigation rates.

TABLE 3 Parameters of the microbial release model (equation 3) in different fecal materials reported or estimated from data sources

Exptl conditions[a]	a (cm^{-1})	β	Reference
Drip irrigation of dairy manure disk*	0.00693 ± 0.00207	5.957 ± 3.917	42
Mist irrigation of dairy manure disk	0.00124	4.9 ± 1.3	42
Drip irrigation of dairy manure disk*	0.00698 ± 0.00161	6.005 ± 2.058	41
Dairy slurry, runoff-box study, vegetation	0.540 ± 0.022	0.884 ± 0.336	40
Dairy slurry, runoff-plot study, vegetated plots	1.077	0.602 ± 0.262	40
Dairy slurry, runoff-plot study bare plots	2.804	0.727 ± 0.873	40
Deer pellet	0.00685	1.924	This study

[a] *, *Cryptosporidium parvum* oocysts and *Giardia duodenalis* cysts were used. *E. coli* was used for all other conditions.

at the intermediate temperature level (20°C), while concentrations were the lowest at intermediate water contents and at the lowest temperature (4°C) (Fig. 1 and 2). Both temperature and water content could influence *E. coli* die-off in deer pellets. The effect of temperature on bacterial survival is well studied, and it is known that *E. coli* die-off typically occurs faster at high temperatures (31, 32, 50, 55, 65, 66). The effect of water content is less well understood, and as of now, there is no general agreement on its effect on microbial inactivation rates. Sinegani and Maghsoudi (52) reported that *E. coli* contents in soil treated with different manure amendments were the highest at permanent wilting point, the lowest at saturation, and intermediate at field capacity. In contrast, Sjogren (67) observed the longest *E. coli* survival in soil microcosms (up to 23.3 months) under saturated moisture conditions. Drying-wetting settings also can influence the *E. coli* survival. Antheunisse and Arkesteijn-Dijksman (68) obtained higher *E. coli* inactivation rates in a filter paper disc study under fast drying than slow drying conditions, while Himathongkham et al. (50) observed lower *E. coli* O157:H7 GFP inactivation rates in the top layer of manure, which dried faster than the middle-bottom manure layer. In our study, the *E. coli* inactivation rates during the second stage of bacterial survival did not differ significantly despite differences in incubation temperature and pellet moisture content (Fig. 2). Similarity in *E. coli* inactivation rates observed at the three temperatures resulted in longer survival and overall higher *E. coli* concentrations at 20°C, primarily due to considerably higher growth rates at 20°C than at 4°C and 35°C. This finding is supported by the results of the *E. coli* survival study conducted in suspensions of bovine or raccoon feces in creek water at different temperatures (69). The author observed a 4-log increase in *E. coli* population within the first 3 days followed by nearly a constant concentration of *E. coli* during 4 consecutive days of incubation. *E. coli* growth was not observed or was insignificant in the suspensions incubated at 0°C, 10°C, and 50°C in that study.

Comparison of *E. coli* survival rates at different temperatures and environments showed that the magnitude of the temperature effect on *E. coli* survival in deer pellets differed from that in other environments (Fig. 3). Specifically, warm-weather conditions extended survival duration, while low temperatures shortened the survival of *E. coli* in deer pellets more than in the other environmental media examined. This further highlights the need for caution when one is predicting the fate and transport of microorganisms associated with white-tailed deer. Using parameters of *E. coli* survival obtained for media other than deer fecal material in fate and transport models is likely to produce misleading results.

There is another concern regarding the use of results and parameters of bacterial survival studies for long-term predictions of microbial fate and transport on large (i.e., watershed) scales. Despite a large number of publications on factors affecting *E. coli* growth and die-off, there are no standard protocols for survival experiments and data processing. Experiments are typically carried out in a laboratory with controlled conditions, such as moisture content, temperature, pH, exposure to UV light, etc., or in uncontrolled field conditions with temporally and spatially variable microbial inputs and growing conditions. Different experimental scales, conditions, bacterial strains, and medium preparation make results of survival experiments difficult to compare and introduce uncertainty into the model parameter estimates, which leads to uncertainty in the model predictions that is difficult to evaluate. Pachepsky et al. (25) have pointed out that the uncertainty in model inputs does not preclude modeling of microbial fate and transport; however, it needs to be factored into simulations. Therefore, new side-by-side experiments with different fecal materials under the same environmental conditions, as well as experiments with the same material under different environmental conditions, are needed to evaluate the variability in bacterial survival and to account for it in the model parameters.

Release experiment. In order to explore how release of *E. coli* from deer pellets compares with *E. coli* release from other sources, we summarized the values of the two parameters, a and β, of the release model (equation 3) obtained in earlier microbial-release studies (Table 3). In our earlier work, we demonstrated that the parameter a can be used as a characteristics of the erodibility for the *E. coli* source material (40) and that it is influenced by several factors, including manure properties, raindrop energy, and salinity of irrigation water. The larger the a values, the faster the applied manure is depleted of *E. coli*. The values of a in studies by Bradford and Schijven (41) and Schijven et al. (42) were lowest in mist irrigation, intermediate in drip irrigation, and highest in sprinkle irrigation applied to bare soil plots. This order coherently indicates the increasing manure erodibility caused by increasing raindrop energy in the order mist, drip, and sprinkle irrigation. The value of a in our study was very close to the values estimated from drip irrigation by Bradford and Schijven (41) and Schijven et al. (42). Therefore, we can surmise that deer pellets have erodibility similar to that of the dairy manure disks used in their studies. Note that *Cryptosporidium parvum* oocysts and *Giardia duodenalis* cysts were used in the studies by Bradford and Schijven (41) and Schijven et al. (42) and that to convert the values of a from cm^{-3}, used in model fitting in this study, to cm^{-1}, used in previous studies, we assumed that the contact area of water drops with deer pellets was approximately 0.07 cm^2. The parameter β in equation 3 determines the shape of the release curve and may vary greatly for the same manure types (Table 3). The value of β for the deer pellets in our study was smaller than those for dairy manure disks and larger than those for dairy slurry.

To illustrate the effect of parameters a and β on the *E. coli* release curves, we plotted the relative cumulative number of released microbial cells (M/M_0) as a function of time for the unit water application rate and the parameter values shown in Table 3 (Fig. 6). As expected, the release of microorganisms from drip-irrigated manure was much slower than that from the sprinkle-irrigated manure slurries, while the release from deer pellets of this study was intermediate between drip and sprinkle irrigation. Based on data published by Laws (70), water drops reached approximately 34% of terminal velocity in this study. This implies that the release of *E. coli* from pellets on non-vegetation-bearing surfaces during real rainfall events can be much faster than that observed under the laboratory conditions of our study.

On vegetation-bearing surfaces undergoing sprinkle irrigation or intensive rainfalls, the *E. coli* organisms released with fecal colloids from fecal deer deposits can be splashed onto leaf surfaces, where they can survive for a long period of time. Islam et al. (71) reported *E. coli* O157:H7 survival in lettuce and parsley for up to 77 and 177 days, respectively. Current regulations in some parts of the country require the establishment of no-harvest buffer zones with minimally a 5-foot radius around spots contaminated by animal intrusion (72). However, these spots can be difficult to identify due to decomposition of the fecal material and washing

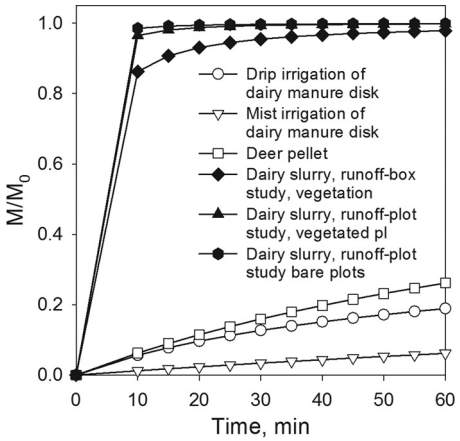

FIG 6 Hypothetical cumulative curves of bacterial release from manure computed with parameter values shown in Table 3.

off with rainfall or irrigation water, while pathogenic bacteria splashed from unidentified spots can still persist in leafy greens. Therefore, preharvest and daily harvest assessments of animal hazards may turn out to be not very efficient in preventing the contamination of leafy greens.

It should be noted that the values of the release parameters shown in Table 3 are estimated for fresh manure material and thus are likely to be different from the values in aged deposits. Several studies reported that fecal coliform (FC) concentrations in the released suspension change with manure age (34–36). Specifically, Kress and Gifford (36) found that peak FC concentrations in suspensions released from standard cattle cowpats declined with age of fecal material. They attributed the decrease to progressive FC leaching from the fecal deposits during recurrent rainfalls. Springer et al. (34) and Thelin and Gifford (35) reported similar decreases in the deposits subjected to rainfall, but they also observed an increase in the FC concentrations released from deposits not subjected to rainfall as their age increased from 2 to 5 days. They explained the observed differences in the released FC concentrations by the kinetics of FC growth and die-off in fecal deposits. By analyzing the effect of the age of artificial dairy cowpats on *E. coli* concentrations in runoff, Muirhead et al. (37) observed an increase in concentration of bacteria in runoff water during the first 2 weeks caused by *E. coli* growth in the deposits.

Based on the growth kinetics of the *E. coli* and the relatively high water content in the deer pellet measured within the first 4 to 8 days of the incubation in our study (Fig. 1 and 2), one can expect the maximum released concentration of *E. coli* to occur at the end of the bacterial growing stage at 20°C. This was the reason for using 4-day-aged feces in this study. However, it remains to be seen whether and to what extent the age, water content, and initial bacterial concentration affect *E. coli* release kinetics and parameters of the release model in deer pellets.

Result of this study indicated that the risk of microbial release and transport from deer feces is maximal in warm and wet weather conditions. The climate of the Midwest region of the United States is getting warmer and more variable. Higher probabilities of hot days (over 32°C), extremely hot days (over 38°C), numbers of days per year with more than 2 in. of rain, and maximum 24-h, 5-day, and 7-day rainfall totals are being expected in the next several decades (73). Increasing temperatures will likely result in faster *E. coli* growth and in overall higher concentrations of *E. coli* in deer fecal deposits compared to those occurring under the current climate conditions. More intensive precipitation will facilitate release of microorganisms from the deposits; therefore, more bacteria can potentially be transported with overland flow and cause further impairment of water bodies. To address the growing risks, reliable information about microbial growth and release from livestock and wildlife deposits is imperative. A lack of such information hinders the progress in development of better management practices for landscapes with mixed agricultural and wildlife use.

Conclusions. Our study represents the first report of *E. coli* survival and release in feces of white-tailed deer. Previous survival studies were conducted mostly with feces of domestic animals, manure-amended soils, sediment, and water, while previous *E. coli* release studies were conducted only with artificial cowpats and manure slurry. Parameters of the survival model obtained for *E. coli* in this study substantially differed from those obtained with other source materials, e.g., feces of domestic animals and manures. This emphasizes the necessity of comprehensive studies of survival and release of naturally occurring populations of microorganisms in wildlife animal feces in order to achieve better predictions of microbial fate and transport in fragmented agricultural and forest landscapes and for development of better management practices to prevent impairment of bodies of water by microorganisms associated with wildlife. The results of the current study suggest that warm wet weather conditions enhance growth and facilitate release of *E. coli* from deer feces into the environment. This is especially important with respect to expected climate changes in the Midwest region of the United States.

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